

USSN 08/422,548
Amendment Under 1.312

Amendments to the Specification

Title

Please replace the title with the following rewritten title:

AN ANTIBODY THAT BINDS TO MPL LIGAND

Sequence Listing

Please delete the paper copy of Sequence Listing filed and replace it with the substitute Sequence Listing filed on February 20, 1996.

Please replace the paragraph beginning at page 19, line 19 with the following rewritten paragraph:

Fig. 9. shows deduced amino acid sequence of human *mpl* ligand (h-ML) (SEQ ID NO: [[1]]6 and human erythropoietin (h-epo) (SEQ ID NO: [[6]]7). The predicted amino acid sequence for the human *mpl* ligand is aligned with the human erythropoietin sequence. Identical amino acids are boxed and gaps introduced for optimal alignment are indicated by dashes. Potential N-glycosylation sites are underlined with a plain line for the h-ML and with a broken line for h-epo. The two cysteines important for erythropoietin activity are indicated by a large dot.

Please replace the paragraph beginning at page 19, line 27, with the following rewritten paragraph:

Fig. 10. shows the nucleotide sequence: coding (SEQ ID NO.: [[7]]8) and deduced amino acid sequence (SEQ ID NO: [[8]]9) of murine *mpl* ligand cDNA. Nucleotides are numbered at the beginning of each line. Amino acid residues are numbered above the sequence starting at Ser 1 of the mature *mpl* ligand (ML) protein sequence. The potential N-glycosylation sites are underlined. Cysteine residues are indicated by a dot above the sequence.

Please replace the paragraph beginning at page 19, line 34 with the following rewritten paragraph:

Fig. 11. shows deduced amino acid sequence of mature human *mpl* ligand (hML) (SEQ ID NO: [[1]]6) and murine *mpl* ligand (mML) (SEQ ID NO: [[8]]10). The predicted amino acid sequence for the human *mpl* ligand is aligned with the murine *mpl* ligand sequence. Identical amino acids are boxed and gaps introduced for optimal alignment are indicated by dashes.

Please replace the paragraph beginning at page 34, line 6 with the following rewritten paragraph:

(7) The amino-terminal sequence of the 18,000, 28,000 and 31,000 bands is the same – SPAPPACDPRLLNKLLRDDHVHLHGR (SEQ ID NO: [[8]]30); and

Please replace the paragraph beginning at page 40, line 15 with the following rewritten paragraph:

- 1) 30 kDa
1 5 10 15 20 25
(S) P A P P A(C)D P R L L N K L L R D D (H/S) V L H (G) R L (SEQ ID NO: [[9]]11)
- 2) 28 kDa
1 5 10 15 20 25
(S) P A P P A X D P R L L N K L L R D D (H) V L (H) G R (SEQ ID NO: [[10]]12)
- 3) 18 kDa
1 5 10
X P A P P A X D P R L X (N) (K) (SEQ ID NO: [[11]]13)

Please replace the paragraph beginning at page 41, line 20 with the following rewritten paragraph:

Based on the amino-terminal amino acid sequence obtained from the 30 kDa, 28 kDa and 18 kDa proteins (see above), two degenerate oligonucleotide primer pools were designed and used to amplify porcine genomic DNA by PCR. It was reasoned that if the amino-terminal amino acid sequence was encoded by a single exon then the correct PCR product was expected to be 69 bp long. A DNA fragment of this size was found and subcloned into pGEMT. The

sequences of the oligonucleotide PCR primers and the three clones obtained are shown in Example V. The amino acid sequence (PRLLNKLLR [SEQ ID NO: [[12]]14]) of the peptide encoded between the PCR primers was identical to that obtained by amino-terminal protein sequencing of the porcine ligand (see residues 9-17 for the 28 and 30 kDa porcine protein sequences above).

Please replace the paragraph beginning at page 42 line 1 with the following rewritten paragraph:

5' GCC-GTG-AAG-GAC-GTG-GTC-GTC-ACG-AAG-CAG-TTT-ATT-TAG-GAG-TCG 3'
(SEQ ID NO: [[13]]15)

Please replace the paragraph beginning at page 89, line 14, with the following rewritten paragraph:

- 1) 30 kDa (1.8 pmol)
1 5 10 15 20 25
(S) P A P P A(C)D P R L L N K L L R D D (H/S) V L H (G) R L (SEQ ID NO: [[9]]11);
- 2) 38 kDa (0.5 pmol)
1 5 10 15 20 25
(S) P A P P A X D P R L L N K L L R D D (H) V L (H) G R (SEQ ID NO: [[10]]12);
and
- 3) 22 kDa (0.5 pmol)
1 5 10
X P A P P A X D P R L X (N) (K) (SEQ ID NO: [[11]]13)

Please replace the paragraph beginning at page 90, line 30 with the following rewritten paragraph:

mpl 1 5' CCN GCN CCN CCN GCN TGY GA 3' (2,048-fold degenerate) (SEQ ID NO: [[14]]16)

Please replace the paragraph beginning at page 90, line 33, with the following rewritten paragraph:

mpl 2 5' NCC RTG NAR NAC RTG RTC RTC 3' (2,048-fold degenerate) (SEQ ID NO: [[15]]17)

Please replace the paragraph beginning at page 91, line 16 with the following rewritten paragraph:

TGACCACGTT CAGCACGGC 69 (SEQ ID NO: [[16]]18)
ACTGGTGCAA GTCGTGCCG (SEQ ID NO: 17)

Please replace the paragraph beginning at page 91, line 24, with the following rewritten paragraph:

CGACCACGTC CATCACGGC 69 (SEQ ID NO: [[18]]19)
GCTGGTGCAG GTAGTGCCG (SEQ ID NO: 19)

Please replace the paragraph beginning at page 91, line 28 with the following rewritten paragraph:

P R L L N K L L R (SEQ ID NO.

[[12]]14)

5' CCAGCACCGCCGGCATGTGACCCCCGACTCCTAAATAAACTGCTTCGTGACG
3' GGTCGTGGCGGCCGTACACTGGGGCTGAGGATTATITGACGAAGCACTGC

Please replace the paragraph beginning at page 91, line 33 with the following rewritten paragraph:

ATCATGTCTATCACGGT 3' (SEQ ID NO: 20)
TAGTACAGATAGTGCCA 5' (SEQ ID NO: 21)

Please replace the paragraph beginning at page 92, line 12 with the following rewritten paragraph:

5' GCC-GTG-AAG-GAC-GTG-GTC-GTC-ACG-AAG-CAG-TTT-ATT-TAG-GAG-TCG 3'
(SEQ ID NO: [[13]]15)

Please replace the paragraph beginning at page 93, line 13 with the following rewritten paragraph:

Forward primer: 5' GCT AGC TCT AGA AAT TGC TCC TCG TGG
TCA TGC TTC T 3' (SEQ ID NO: [[22]]21)

Reverse primer: 5' CAG TCT GCC GTG AAG GAC ATG G 3' (SEQ ID NO: [[23]]22)

Please replace Table 3, beginning at page 99, line 25, with the following rewritten Table:

Table 3.

Peptide Fragments Used for Total Synthesis of h-ML Using Ligase

Fragment	Sequence
1 (SEQ ID NO: [[24]] <u>23</u>)	iNOC-HN-SPAPPACDLRVLSKLLRDSHVVLH-glc-K-NH ₂ (1-23)
2 (SEQ ID NO: [[25]] <u>24</u>)	iNOC-HN-SRLSQCPEVHPLPTPVLLPAVDF-glc-K-NH ₂ (24-46)
3 (SEQ ID NO: [[26]] <u>25</u>)	iNOC-HN-SLGEWKTQMEETKAQDILGAVTL-glc-K-NH ₂ (47-69)
4 (SEQ ID NO: [[27]] <u>26</u>)	iNOC-HN-LLEGVMAARGQLGPTCLSSL-glc-K-NH ₂ (70-89)
5 (SEQ ID NO: [[28]] <u>27</u>)	iNOC-HN-LGQLSGQVRLLL GALQSL-glc-K-NH ₂ (90-107)
6 (SEQ ID NO: [[29]] <u>28</u>)	iNOC-HN-LGTQLPPQGRRTAHKD PNAIF-glc-K-NH ₂ (108-128)
7 (SEQ ID NO: [[30]] <u>29</u>)	H ₂ N-LSFQHLLRGKVRFLMLVGGSTLCVR-CO ₂ (129-153)